

Article Addendum

Plant Parasitic Oomycetes Such as *Phytophthora* Species Contain Genes Derived from Three Eukaryotic Lineages

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ABSTRACT

Fungi and the oomycetes include several groups of plant pathogenic microbes. Although these two eukaryotic groups are unrelated they have a number of phenotypic similarities suggested to have evolved convergently. We have recently shown that gene transfer events have occurred from fungi to the oomycetes. These gene transfer events appear to be only one part of a complex and chimeric ancestry for the oomycete genome, which has also received genes from a red algal endosymbiont.

As genomic sampling increases, a persistent pattern of horizontal gene transfer (HGT) between microbial lineages is becoming evident.^{1,2} So far, patterns of horizontal gene transfer have been identified in four main forms: (A) gene transfer between prokaryote lineages, such that a large proportion of many prokaryote genomes are likely to be chimeric,^{3,4} (B) gene transfer from the prokaryote progenitors of the mitochondrion and the plastid organelles to a host eukaryote nuclear genome (e.g., refs. 5–7), (C) gene transfer from prokaryote genomes to eukaryote microbes, often involving phagocytic eukaryotes and microbes that share similar habitats⁸ and (D) gene transfer from a eukaryotic endosymbiont to their host eukaryotic genomes.^{9,10} This fourth form of gene transfer includes secondary and tertiary endosymbiotic events and has so far provided our best examples of eukaryote-to-eukaryote gene transfer.^{11,12} Secondary and tertiary endosymbiotic events are typified by the engulfment of a photosynthetic eukaryote by another eukaryote followed by the reduction of the consumed photosynthetic eukaryote and transfer of genes from the endosymbiont to the host nuclei with some retargeting of the transferred gene products back to the remnant organelle.^{9,10}

Gene transfer events can be identified using phylogenetic analysis when an individual gene tree topology contradicts a known species relationship. HGT can only be seriously considered, however, if the gene phylogeny shows that the putative HGT is nested within a donor clade with strong bootstrap support.² Endosymbiosis typically leads to multiple cases of nuclear-encoded genes demonstrating endosymbiotic ancestry, with the candidate genes grouping within a clade representing the lineage that gave rise to the progenitor of the endosymbiont.⁵ There have been multiple cases of both secondary and tertiary endosymbiosis within the eukaryotes, making the evolutionary reconstruction of phototrophy in the eukaryotes highly complex.⁹ Secondary and tertiary endosymbiotic remnant organelles are often identified by the presence of three or more membranes surrounding the organelle body.¹⁰ However, secondary endosymbiotic events have led to a range of different combinations of cell apparatus, from the total loss of the endosymbiont-derived organelle^{13,14} to the maintenance of the organelle compartment¹⁰ and the possession of a remnant nucleus as a nucleomorph.¹⁵

The oomycetes include the plant pathogenic *Phytophthora* spp. and are heterokonts (sometimes called Stramenopiles).¹⁶ The heterokonts also encompass numerous groups of photosynthetic algae (e.g., *Bolidomonas*, Diatoms, Xanthophyceae, Phaeophyceae and Chrysophyceae) and are proposed to be derived from an ancestrally photosynthetic cell that obtained its plastid by engulfment of a red alga.¹⁶ Cytological studies of the oomycetes have so far failed to identify a relic plastid organelle but the recent publication of the *Phytophthora sojae* and *Phytophthora ramorum* genomes identified 855 genes putatively originating from the genome of a photosynthetic microbe consistent with a phototrophic ancestry for the oomycetes.¹³

Phytophthora plant pathogens include the causal agents of sudden oak death (*P. ramorum*), potato blight (*P. infestans*) and, *P. sojae* which causes serious root and stem rot of soybean plants. Initially, *P. infestans* was identified as a fungal pathogen and the causal agent of the great 1845 Irish potato famine by Rev. Miles J. Berkeley,¹⁷ due to

life cycle similarities and an apparently homologous mode of plant infection to ascomycete plant pathogens. It was only with the use of molecular phylogenetic methods starting with small subunit rDNA analysis¹⁶ followed by multiple concatenated gene phylogenies¹⁸ that the oomycetes were demonstrated to group within the heterokont radiation. With the apparent phylogenetic origins of the oomycetes pinpointed it left the apparent similarities in pathogenic mechanism and infective lifecycle between the filamentous ascomycetes and the oomycetes a mysterious case of convergent evolution.¹⁹

During the evolutionary analyses of the predicted proteome of the filamentous plant pathogenic ascomycete *Magnaporthe grisea*²⁰ we detected a series of unexpected similarities in the genomes of plant pathogenic ascomycetes and the oomycete genomes.¹³ We followed up this observation by further investigation using phylogenetic methods combined with comparative genomic analysis, which revealed a series of HGT events. We subjected our datasets to a range of tests: (A) to test that the level of support for the tree topology seen was robust given random resampling of the sequence alignments used to reconstruct the gene phylogenies; (B) to ensure that the possibility that similar topologies with the oomycete/filamentous ascomycete relationship removed could be rejected at the 0.05 confidence level and; (C) to test for alternative patterns of gene evolution including hidden paralogy (duplication with differential patterns of gene loss) were unlikely. Four of the datasets tested in this way held up to our scrutiny and were thus proposed as fungi-to-oomycete horizontal gene transfers.²¹ The predicted function of three of the four genes (*CodB*, a purine permease, *AraJ*, a sugar transporter and a *PcaH* an extracellular dioxygenase) could conceivably be useful for an osmotrophic microbe living in a plant associated habitat (biotrophy), suggesting that these HGT events could in-part explain the convergently evolved similarities in osmotrophy and filamentous growth habit seen in the oomycetes and fungi. Our analyses also suggested that three of these HGTs originated from a genome closely related to the last common ancestor of the *Magnaporthe* and *Aspergillus* evolutionary branches. Although the specific branching position of the transferred lineage could not be pinpointed in the fourth analysis, the same point of origin could not however be excluded. This suggests that the four HGTs we identified could be derived from the same source, a phenomenon similar in pattern (if not involving the same lineages) to that seen for phylogenetic tree topologies used to investigate the endosymbiotic events discussed above. Although these analyses do not shed any light on the circumstances in which these transfers occurred, it is possible that an intimate association between a fungus and a heterokont has led to genetic exchange and demonstrates that eukaryote-to-eukaryote gene transfers are not just associated with the acquisition of phototrophy by secondary/tertiary endosymbiosis.

Our published study was conducted using only published genome sequences as a seed for comparative genomic analyses.²¹ However, with the very recent publication of two *Phytophthora* genomes¹³ it is possible that further analyses will identify additional candidate *Phytophthora*-Fungi HGT events when they are carried out. These tests may determine how pervasive the pattern of HGT is within the oomycetes.

The oomycetes have been classified within the phylum Pseudofungi¹⁶ which comprises a number of microbial lineages with phenotypic similarities to true fungi, including hyphae-like structures and osmotrophy. Originally, the term Pseudofungi was used to group together 'water-moulds' possessing mastigonemes (tubular tri-partite hairs) on one flagellum. Currently the phylum

Pseudofungi comprises the biotrophic oomycetes including parasites of plants and brown algae, the phagotrophic *Devolpayella* and the biotrophic hyphochytrids, including the diatom ectoparasite *Pirsonia*.¹⁶ It will be interesting to ascertain at what point within the diversification of the Pseudofungi the HGTs that are identified²¹ became fixed and how the acquisition of these phenotypes relates to the evolution of Pseudofungi phenotypes within the heterokonts. Independent of the specific ancestry of the gene transfer events within the Pseudofungi it is clear that *P. sojae* and *P. ramorum* have chimeric genomes, originating from three separate eukaryotic lineages, the ancestral heterokont nuclear genome, the red algal endosymbiont and at least four genes of fungal ancestry donated to an oomycete nuclear genome.

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